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Tau protein hyperphosphorylation in children with cerebral palsy with sleep disorders

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ABSTRACT

Aims: In this study, we aimed to evaluate the relationship between sleep disorder and serum tau protein levels in children with cerebral palsy.

Methods: The sample was selected among children aged 6-10 years who applied to our physical medicine and rehabilitation outpatient clinic. In order to evaluate sleep quality, the Pittsburgh Sleep Quality Index (PSQI) questionnaire was recorded by asking parents of all participants. Children with CP who had a Pittsburgh Sleep Quality Index value above 5 were determined as the sleep disorder group. We recruited 27 children with CP and sleep disorders in the first patient group (Group 1), 27 children with CP but without sleep disorders in the second group (Group 2). The third group (Group 3), which was the healthy group, included 27 children without any disease. We also recorded the age of children at diagnosis, risk factors for CP (premature, prolonged birth, etc.), CP type, gross motor function classification system (GMFCS), botox application, orthoses usage, maternal age at birth, and additional problems. We measured total tau protein (T-tau) and phosphorylated tau protein (P-tau) levels in blood samples through a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). The correlation between tau protein and PSQI values was examined.

Results: Maternal age (p=0.001), gross motor function classification system (GMFCS) (p=0.001), and pittsburgh sleep quality index (PSQI) (p=0.001) were significantly higher in the group with sleep disorders. There was a statistically significant difference between the groups in terms of serum T-Tau and P-tau protein levels (T-tau p=0.003, P-tau p=0.004). In the group sleep disorders, PSQI was significantly correlated with T-tau (r=0.499) and P-tau (r=0.473).

Conclusion: This study shows that tau protein levels are higher in CP patients with sleep disorders than in participants without sleep disorders. In the correlation analyzes, a positive and significant correlation was observed between PSQI values and T-tau and P-tau in sleep disorders groups, and no correlation was found in without sleep disorders.

Keywords: Cerebral palsy, Tau protein, phosphorylated tau protein, sleep disorders

INTRODUCTION

Cerebral palsy (CP) is defined as a group of persistent disorders characterized by non-progressive impairments in movement and posture development occurring in the developing fetal or infant brain.[1] It is shown to occur between 1.5-3 per 1000 live births worldwide and 4.4 in Turkey.[2] The etiology of CP bears multiple risk factors; the prevalent causes are intraventricular bleeding, periventricular leukomalacia, bronchopulmonary dysplasia, intrauterine growth retardation, intrauterine infections, antepartum bleeding, severe placental pathologies, and multiple pregnancies. Ultimately, such factors directly or indirectly trigger hypoxic brain damage, leading to neuronal losses in the pathogenesis of CP.[3,4] Besides, many comorbidities (vision, hearing, or cognitive impairment and epilepsy) often accompany motor disorders in CP.[5] Sleep problems are also a common phenomenon in this population at 23-50% and are probably triggered by the above comorbidities.[6-9] Long-term sleep disorders in children may lead to neuronal losses. Additional neuronal losses due to sleep disorders to the already existing upper motor neuron damage are likely to bring unprecedented negativities to a child with CP.

Tau protein forms polymers of the cytoskeleton mainly in the axons of the central nervous system. Its major functions may be listed as ensuring the formation and coupling of microtubules, maintaining the structure and stability of neurons, and mediating the transport of intracellular microvesicles.[10] It is also a neuronal skeletal protein joining actin filaments made up of neurons. In general, tau protein is released in the case of any neuronal damage; therefore, the degree of neuronal damage is

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measured by identifying serum or CSF levels of tau protein.[11] Tau protein appears in two forms: dephosphorylated and phosphorylated. In the progression of hypoxia, tau protein becomes excessively phosphorylated, affecting regular physiological functions. Tau protein accumulates in neurons, altering normal microtubule formation. In addition, it separates normal microtubule-linked proteins from the microtubule, causing microtubule collapse and generating large amounts of accumulated neuron-damaging matter.[12,13]

Tau protein hyperphosphorylation is often demonstrated in Alzheimer's disease, traumatic brain injury, and acute ischemic stroke.[14] We believe that Tau and phosphorylated Tau protein molecules have a significant role in elaborating on the etiology of sleep problems, a common condition among those with CP at 23-50%. In this study, we aimed to measure serum tau protein levels in relation to existing brain damage in CP patients with sleep disorders.

METHODS

The study was carried out with the permission of Hitit University Medical Faculty Clinical Researches Ethics Committee (Date: 19.08.2020, Decision No: 2020/171). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In this study, 54 children with CP and 27 healthy children were included, which was carried out at Hitit University Faculty of Medicine, Department of Physical Medicine and Rehabilitation. The sample was selected among children aged 6-10 years. Children with CP who had a Pittsburgh Sleep Quality Index value above 5 were determined as the sleep disorder group. Accordingly, we recruited 27 children with CP and sleep disorders in the first patient group (Group 1), 27 children with CP but without sleep disorders in the second group (Group 2). The third group (Group 3), which was the healthy group, included 27 children without any disease. We also recorded the age of children at diagnosis, risk factors for CP (premature, prolonged birth, etc.), CP type, gross motor function classification system (GMFCS), botox application, orthoses usage, maternal age at birth, and additional problems. We obtained informed consent from the parents and children for voluntary participation in the study. Patients between the ages of 6 and 10 who were diagnosed with CP and who did not have a history of genetic, epilepsy, metabolic disease or mental retardation were included in this study. A nurse took the blood samples of the patient and control groups into 8 mL clot activator tubes in our polyclinic between 08.00 and 10.00 after 12 hours of fasting. After keeping the samples in the tubes at room temperature for half an hour, they were centrifuged at 4,000 g for 10 minutes, and then 4 mL of serum was obtained from each. The serum samples separated into eppendorfs were stored at -70°C until the analysis.

Tau and phosphorylated tau protein levels: We measured tau protein and phosphorylated tau protein levels in blood samples through a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). In this method, we added the samples to plate wells coated with monoclonal antibodies and incubated them. Following incubation, an immune complex was formed with a biotin-labeled antibody and streptavidin-HRP solution. Unbound protein and enzymes were washed from the medium. Then, chromogenic reagent A and B solutions were added and incubated at 37°C for about 10 minutes in an environment away from light. Finally, the stop solution was added, and the optical density was determined at a wavelength of 450 nm within 10 minutes.

Pittsburgh Sleep Quality Index (PSQI): The PSQI was developed in 1989 to evaluate sleep quality and adapted into Turkish by Agargün et al.[15] The instrument includes 24 questions within 10 components and measures sleep quality over a 1-month time interval. One may obtain a maximum score of 21 points, and a score greater than 5 indicates poor sleep quality.

Statistical Analysis

While nominal and ordinal data were presented as frequencies and percentage, we showed the continuous data as means and standard deviations. The categorical variables were compared between the groups using Chi-Square, Likelihood Ratio, and Fischer's Exact tests. Besides, we tested the normality of distribution using the Kolmogorov-Smirnov test. Accordingly, while non-normally distributed variables were subjected to Kruskal Wallis-H and Mann-Whitney U tests, we performed independent samples t-test and one-way analysis of variance (ANOVA) to test the variables showing a normal distribution. Moreover, we tested the association between our main variables using Spearman's rho correlation analysis. We performed all statistical analyses on SPSS 17.0 at a 95% confidence interval, and a p-value < 0.05 was considered significant.

RESULTS

Our study included 27 CP patients with sleep disorders, 27 CP patients without sleep disorders, and 27 healthy volunteers. The mean age of the participants was 7.89±1.42. There were 24 (29.6%) female and 57 (70.4%) male participants. There was no difference between the groups in terms of gender and age (p=0.072, p=0.110). The findings revealed that the groups significantly differed by CP type. Accordingly, spastic CP was significantly more common in the group 1 (p=0.001). Speech disorder (p=0.033), hearing disorder(p=0.041) and vision defect (p=0.007) were significantly more common in the group 1. Moreover, we found maternal age (p=0.001) and GMFCS (p=0.001) were significantly higher in the group 1. No statistically significant difference was found between the SP groups in

terms of orthosis use (**Table 1**). Mean serum T-tau protein level was 11.19±7.12 ng/ml (min-max: 5.41-36.56) ng/ml in group 1, 8.79±3.59 ng/ml (min-max: 4.78-14.9) in Group 2 and 7.04±2.62 ng/ml (min-max: 0.47-14.7) in group 3. Mean serum P-tau protein level was 136.85±88.7 pg/ml (min-max: 71.9-495) in group 1, 105.79±29.20 pg/ml (min-max: 74.11-151.5) in Group 2 and 88.51±12.27 pg/ml (min-max: 67.9-112.5) in group 3. There was a statistically significant difference between the groups in terms of serum T-Tau and P-tau protein levels (T-tau p=0.003, P-tau p=0.004), and this difference was found between Group 1 between Group 2 (T-tau and P-tau p=0.001) and Group 1 between Group 3(T-tau and P-tau p=0.001). No statistically significant difference was found between Group 2 and Group 3 in terms of serum T-tau and P-tau protein levels (T-tau p=0.06, P-tau p=0.062) (**Table 2**). T-tau and P-tau levels are shown in **Figures 1** and **2**. In the correlation analyzes, a positive and significant correlation was observed between PSQI values and T-tau (r=0.499; p < 0.05) and P-tau (r=0.473; p < 0.05) in Group 1, and no correlation was found in Group 2 and Group 3 (**Table 3**).

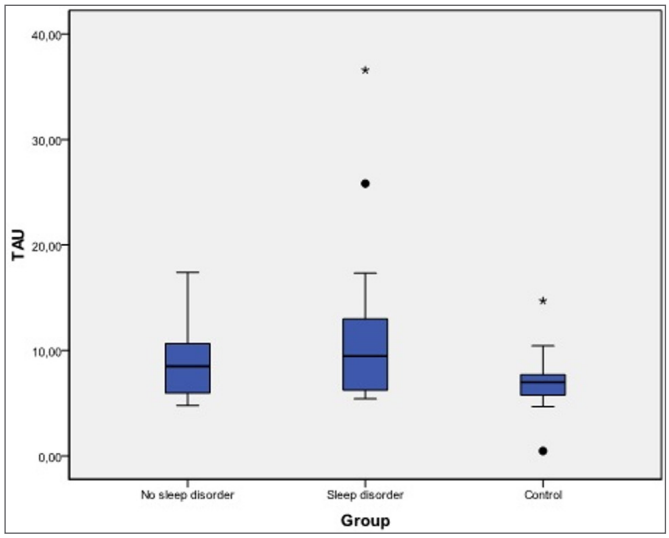

Figure 1: Tau protein levels by groups

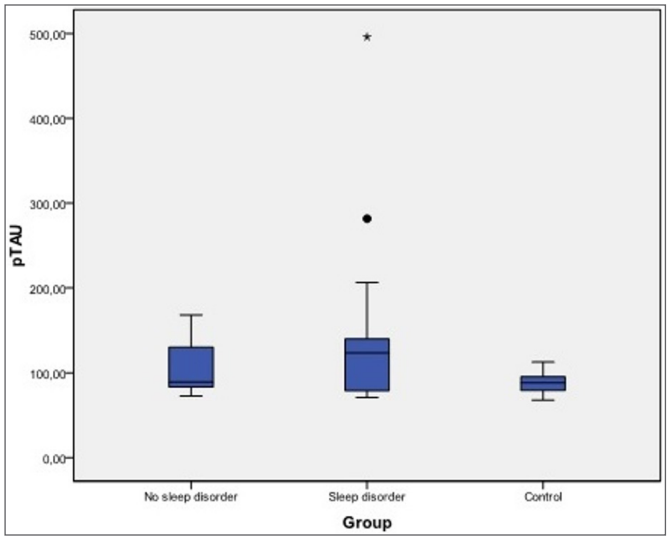

Figure 2: Phosphorylated tau protein levels by groups

Table 1. Comparison of demographic and clinical data of groups

	Group 1 (n=27)	Group 2 disorder (n=27)	Group 3 (n=27)	p
Age, mean±SD	7.48±1.42	8.00±1.66	8.32±1.36	0.110[a]
Sex, n (%)				
Female	5 (18.5)	9 (33.3)	10 (35.7)	0.072[b]
Male	22 (81.5)	18 (66.7)	18 (64.3)	
CP type, n (%)				
Spastic	20 (74.1)	9 (33.3)		0.001[b]
Hypotonic	5 (18.5)	9 (33.3)		0.005[b]
Dyskinetic	2 (7.4)	4 (14.8)		
Ataxic	-	5 (18.5)		
Risk factor, n (%)				
Premature	18 (66.7)	18 (66.7)		0.486[c]
Prolonged birth	9 (33.3)	8 (29.6)		
Postnatal thyroid	-	1 (3.7)		
Speech disorder, n (%)	23 (85.2)	16 (59.3)		0.033[d]
Hearing disorder, n (%)	18 (66.7)	9 (33.3)		0.014[d]
Vision defect, n (%)	19 (70.4)	9 (33.3)		0.007[d]
Orthosis usage, n (%)				
None	7 (25.9)	6 (22.2)		
SOLID AFO	12 (44.4)	14 (51.9)		
KAFO	5 (18.5)	4 (14.8)		0.952[c]
PAFO	3 (11.1)	3 (11.1)		
Botox, n (%)	17 (63.0)	13 (48.1)		0.206[d]
Maternal age	35.00±1.82	29.78±4.20	26.54±4.39	0.001[e]
GMFCS				
1		1 (3.7)		
2	1 (3.7)	6 (22.2)		
3	7 (25.9)	15 (55.6)		0.001[c]
4	12 (44.4)	4 (14.8)		
5	7 (25.9)	1 (3.7)		
PSQI	13.48±3.27	2.59±0.97	2.21±0.83	0.001[a]

a. Kruskal Wallis-H Test, b. Chi-Square Test, c. Likelihood Ratio Test, d. Fischer's Exact Test, e. One Way ANOVA, f. Independent Samples t-test, SD: Standard Deviation, AFO: Ankle foot orthosis, KAFO: Knee foot orthosis, PAFO: powered ankle-foot orthoses, GMFCS: Gross motor function classification system, T-tau: Total tau protein, P-tau: phosphorylated tau protein, PSQI: Pittsburgh Sleep Quality Index

Table 2. Comparison of T-tau and P-tau levels between groups

Groups	T-tau***	p value		P-tau***	p value	
Group 1	11.19±7.12	0.001**		136.85±88.70	0.001**	
Group 2	8.79±3.59	0.001**	0.003*	105.79±29.20	0.001**	0.004*
Group 3	7.04±2.62	0.061**		88.51±12.27	0.062**	

*: Kruskal Wallis analysis of variance; **: Mann Whitney U test, ***: Mean and standard deviation (minimum-maximum), Group 1 was compared with Groups 2 and 3, it was statistically significant at the p=0.001 level; Group 2 and Group 3 were compared, not statistically significant, T-tau: Total tau protein, P-tau: phosphorylated tau protein

Table 3. Spearman's rho correlation analysis between PSQI between T-tau and P-tau for groups

PSQI	Group 1 (n=27)	Group 2 (n=27)	Group 3 (n=27)
T-tau	0.499*	-0.177	0.153
P-Tau	0.473*	-0.058	-0.189

* p< 0.05, T-tau: Total tau protein, P-tau: phosphorylated tau protein, PSQI: Pittsburgh Sleep Quality Index

DISCUSSION

CP represents one of the most common physical childhood disabilities worldwide.[16] Children with CP are a population at risk of developing sleep problems. While the prevalence rates reported by parents range from 23% to 46%, it ranges from 20% to 30% in healthy developing children.[17,18] The previous research showed that children with CP with more severe functional motor limitation, stiffness, and contractures, often characterized by bilateral spasticity, have more severe sleep disorders.[19] In this study, spasticity was found to be significantly higher in the group with sleep disorders. The studies by Romeo et al.[20] (with children with GMFCS level V) and Sandella et al.[21] revealed that GMFCS predicts sleep problems. Overlapping the findings in the literature, we found that the sleep disorders group had significantly higher GMFCS values than the without sleep disorders group.

Many comorbidities, such as vision, hearing or cognitive impairment often accompany motor disorders in CP.[5] Sleep problems are a common phenomenon in this population at 23-50% and are probably triggered by the mentioned CP comorbidities.[6-9] In our study, speech, vision and hearing disorders were significantly higher in the sleep disorders group than in the without sleep disorders group.

Tau is among the essential microtubule-associated proteins in neurons. Balanced phosphorylation binds it to microtubules, maintaining the coupling of microtubules and the structures and stability of neurons. Tau protein brings stability and plastic properties to the neuronal cytoskeleton, facilitating the formation of synaptic networks that underlie essential neurobiological functions.[22] On the other hand, hyperphosphorylation of tau protein leads it to aggregate and form paired helical filamentous structures known as neurofibrillary tangles.[23] The emergence of increased expression of its gene following brain ischemia raises hopes for a better understanding of the roles of tau protein, whose functions are not fully understood in the effects of ischemic diseases.[24] Besides, despite significant progress in recent research on the pathogenicity of tau protein following ischemia, the problems caused by impaired mechanisms resulting from tau protein after ischemia are unclear in children with CP.[7]

Sleep and rest refer to global states of control at all levels of biological organization, including genes, neural circuits, and brain systems.[25] The insomnia loop may be one of the best examples of self-organized operations in neuronal circuits and brain systems, requiring perfect synaptic coordination in multiple cerebral organizations. In mammals, sleep-wake sequencing and timing are regulated by circadian and homeostatic processes that contribute to one's sleep pattern.[26,27] Both processes require coordinated interaction between the suprachiasmatic nucleus and diencephalic structures, basal forebrain, and brainstem.[25] Thus, the emergence of such a complex circuitry requires both stable and plastic properties of the neuronal cytoskeleton promoted by tau protein in the early postnatal period. In addition, it was concluded that microtubules affect circadian activity patterns by modulating the sensitivity of different melatonin receptors.[28] There are many studies related to tau protein in sleep disorders. Evidence from animal models in the study by Di Meco et al.[29] suggested that changes in the sleep-wake cycle may increase levels of hyperphosphorylated tau protein in the brain. Winer and colleagues,[30] assessed associations of tau levels with sleep in older adults, comparing objective (wristwatch actigraphy) and subjective (PSQI) sleep measures over 1 week. Objective and subjective (PSQI) sleep disturbance was associated with higher tau levels. Benedict and et al.[31] study on 15 healthy men observed that serum tau protein levels increased from evening to morning in sleep loss. Work from Holth[32] and Lucey[33] and colleagues, demonstrated > 50% increased cerebral spinal fluid tau in healthy adults (30-60 years old) with one-night of sleep deprivation. As far as we know, our study will be the first study to examine the tau protein level measurement in CP and its relationship with sleep disturbance. In our study, T-tau and P-tau levels were found to be significantly increased in CP patients with sleep disorders. A significant relationship was observed between PSQI values and tau proteins, and taupatology was found to be important in sleep disorder.

There are some limitations to this study. First, we did not consider cerebrospinal fluid tau and phosphor-tau protein levels and neuroimaging in this study. In addition, since the literature does not host a similar study with patients with CP, we could not compare our results fully with what was previously found in the literature. We think that tau protein can be used as a marker as an indicator of sleep dysfunction in patients with CP. But, our findings need to be confirmed by further research.

CONCLUSION

This study shows that tau protein levels are higher in CP patients with sleep disorders than in participants without sleep disorders. In the correlation analyzes, a positive and significant correlation was observed between PSQI values and T-tau and P-tau in sleep disorders groups, and no correlation was found in without sleep disorders.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Hitit University Medical Faculty Clinical Researchs Ethics Committee (Date: 19.08.2020, Decision No: 2020/171).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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